Super-capacity Information-Carrying Systems Encoded by Spontaneous Raman Scattering

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Experimental Section

Materials. Aminomethyl Polystyrene Resin, 1% DVB (loading 0.5-0.7 mmol/g)，Rink Amide MBHA resin 100-200 mesh, 9-fluorenylmethyloxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu), 6-chloro-1-hydroxybenzotriazole (Cl-HOBt), N, N'-diisopropylcarbodiimide (DIC), *o*-benzotriazole-N, N, N', N'-tetramethyl-uronium-hexafluorophosphate (HBTU) and Fmoc-protected amino acids were purchased from GL Biochem (Shanghai, China). N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), dichloromethane (DCM), N, N-diisopropylethylamine (DIEA), methanol (MeOH), N-methylpyrrolidone (NMP), diethyl ether, trifluoroacetic acid (TFA) and polymethyl methacrylate (PMMA) were purchased from Sinopharm Chemical Reagent. All other chemical reagents including triisopropylsilane (TIS), 2-ethynylaniline (**Ⅰ-2097**), 4-ethynylaniline (**Ⅰ-2101**), methyl 4-ethynylbenzoate (**Ⅰ-2109**), 4-ethynylbenzoic acid (**Ⅰ-2110-acid**), 4-((trimethylsilyl)ethynyl)benzoic acid (**Ⅲ-2162-acid**), 4-aminobenzonitrile (**Ⅳ-2215**), 3-phenylpropiolic acid (**Ⅳ-2225-acid**), and methyl 3-phenylpropiolate (**Ⅳ-2228**) were purchased from Aladdin Bio-Chem Technology (Shanghai, China). The peptide bead library was synthesized using TentaGel S-NH_2 resin as the solid support. TentaGel S-NH_2 resin was purchased from Rapp Polymere Gmbh (Tubingen, Germany). Unless noted otherwise, all materials were used without further purification after the purchase from the commercial providers. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrophotometer. The high resolution mass spectra (HRMS) were obtained on a Shimadzu LCMS-IT-TOF mass spectrometer by ESI. U-87MG cells were purchased from Procee Life Science & Technology (Wuhan, China). U-87MG cells were maintained in MEM supplemented with 10% FBS and 1% penicillin-streptomycin. All cells were maintained in a humidified 5% CO_2 incubator at 37℃.

Confocal Raman Spectroscopy and Spectral Analysis. Regular Raman spectra for the encoded beads were obtained using a Renishaw inVia reflex spectrometer (Wotton-under-Edge, UK), operating with a near-infrared diode laser emitting at 785 nm and a thermoelectrically cooled CCD, coupled to a Leica DMLM microscope (50× air objective, NA 0.75). The spectral resolution is 2 cm^{-1}. The calibration of the wavenumber axis was done by recording the Raman spectrum of a silicon wafer (1 accumulation, 10 s) in the static mode. The laser power was 15 mW. When each bead was measured, 5 or 10 frames were collected to average the signals and background noise, where each spectra frame was measured for 2 s exposure. In the library screening experiment, DXR Raman microscope (Thermo Fisher) was used to image cell binding with a 10× air objective (long distance) and measure the spectra of the encoded beads with a 50× air objective (long distance). The power of a 785 nm laser was 24 mW. The spectra of 10 frames were collected and each spectra frame was measured for 5 s exposure. For decoding the 2-D surfaces containing spots of mixed solutions, the same

procedures were taken using the DXR Raman microscope. All the raw spectra were processed using the Thermo Scientific OMNIC™ software by automatic smoothing and baseline correction when needed. The peak of the Raman reporter molecule Ⓡ or R was used to normalize each spectrum as an internal standard. The results were presented using the softwares of Origin 8 and Igor Pro 6.37.

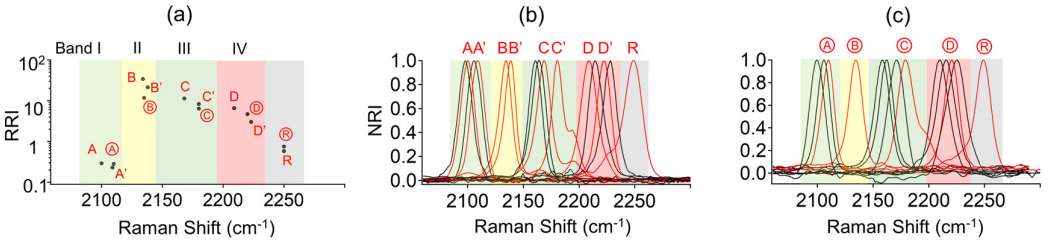


Figure S1. The spectral properties of selected alkyne candidates for super-capacity coding systems. Experimentally, the compounds labeled with A, A', B, B', C, C', D, D' and R, were selected for solution mixing and spotting onto a surface. The compounds labeled with Ⓐ, Ⓑ, Ⓒ, Ⓓ, and Ⓡ, were selected to covalently attach to aminolated resin beads. (a) Relative Raman intensities of the selected compounds. The peak positions of the selected compounds are plotted as spectra of normalized Raman intensities in (b) and (c).

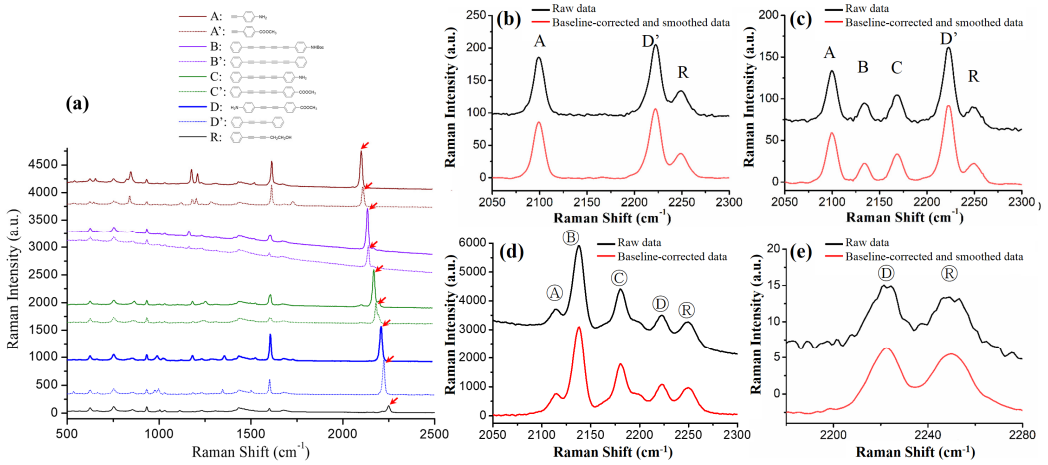


Figure S2. (a) Raw spectra of the coding compounds in NMP. The measured concentrations were 2.6 M for A, 3.3 M for A', 0.026 M for B, 0.033 M for B', 0.057 M for C, 0.075 M for C', 0.10 M for D, 0.27 M for D', and 0.25 M for R. (b)-(e) Examples of raw and processed spectra, representing codes of $A_5D'_5$, $A_5B_3C_4D'_6$, $Ⓐ_3Ⓑ_7Ⓒ_5Ⓓ_4$ and Code #4 in the cell-binding screening experiment, respectively. Spectra have been offset vertically for clarity.

Decoding Analysis of Measured Spectra. As shown in Table S2, logically the decoding analysis would be done by comparing the experimental results of RRI (the coding peak: the reference peak) in the obtained spectra with the designed/expected RRI, and then determining which exact codes the obtained spectra correspond to. However, with some possibility when the

encoding reactions such as mixing or solid-phase synthesis may affect the eventual measured intensities, the more precise and straightforward option than this strategy, is to fabricate all the standard codes and save their spectra for the practical readout/decoding process. We decoded the spectra using this method.

The encoding synthesis was independently done by one researcher, and the decoding measurements/analysis was blindly done by another researcher who didn't communicate with the previous person. Basically the standard codes were first made and measured by Researcher 1. These standards were used for further comparison with the measured spectra of unknown codes by Researcher 2, and then the codes were determined. In the experiment of bead-based screening of cell-binding peptides, Researcher 1 obtained spectra of all the 8 standard codes. After identifying positive and negative beads, by comparing with the standards, Researcher 2 determined the codes of all the positive and negative beads. In the experiment for the Raman-ASCII system to write English words, Researcher 1 fabricated all the 128 ASCII codes and measured them. Then the decoding of the spectra for words "Central China Normal University" was done by Researcher 2 who compared these spectra with the standards. In the experiment for the Raman-Unicode system to write Chinese words, since it was not realistic to make all the 65,536 codes manually in the lab, the experimental spectra for "华中师范大学" were decoded by comparing with the coding unit graph in Figure 2a.

Fabrication of Data Storage Chip

Preparation of the Code Unit Solutions. Blank NMP was used as the coding units A_0 and D_0. 2.6 M compound A and 0.10 M compound D in NMP were diluted to seven concentrations by a factor of 1×, 1.5×, 2.25×, 3.38×, 5.06×, 10.13×, and 20.25×, to obtain the code units A_1-A_7 and D_1~D_7, respectively. 0.27 M compound D' in NMP was diluted to 8 concentrations by a factor of 1×, 1.5×, 2.25×, 3.38×, 5.06×, 6.75×, 10.13×, and 20.25×, respectively to obtain the code units D'_0~D'_7.

Then equal volumes of 0.25 M compound R, $A_{(0\sim7)}$ and $D_{(0\sim7)}$ were mixed combinatorically to produce the coding solutions for the first 64 codes in the ASCII system. The same procedure was take for R, $A_{(0\sim7)}$ and $D'_{(0\sim7)}$ to produce the coding solutions for the second 64 codes in the ASCII system. **Table S1** lists the relationships among the ASCII characters, the octal codes and the corresponding Raman codes. The 128 coding solutions were mixed with same volume of 10% PMMA solution in acetone and used in the next writing step.

Writing Text Using the ASCII-Raman Coding System. The coding solutions for each character in the authors' affiliation "Central China Normal University" were selected for use (see **Table S1** and **Figure S3** or **Figure 4** in the main text). A quartz slide (2.5 cm × 7.5 cm) hydrophobic surface was pretreated by 1% OTS in *n*-hexane. 0.2 μL of the coding solutions

with 10% PMMA solution in acetone (volume ratio = 1 : 1) were spotted onto the surface. After drying in 37℃ oven for 2 h, the coded PMMA films were formed on the surface as a data storage chip.

Table S1. The ASCII characters and octal codes, and their corresponding Raman codes.

OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM
000	NUL	A_0D_0	010	BS	A_0D_1	20	DLE	A_0D_2	30	CAN	A_0D_3	40	Space	A_0D_4	50	(	A_0D_5	60	0	A_0D_6	70	8	A_0D_7
001	SOH	A_1D_0	011	TAB	A_1D_1	21	DC1	A_1D_2	31	EM	A_1D_3	41	!	A_1D_4	51	)	A_1D_5	61	1	A_1D_6	71	9	A_1D_7
002	STX	A_2D_0	012	LF	A_2D_1	22	DC2	A_2D_2	32	SUB	A_2D_3	42	"	A_2D_4	52	*	A_2D_5	62	2	A_2D_6	72	:	A_2D_7
003	ETX	A_3D_0	013	VT	A_3D_1	23	DC3	A_3D_2	33	ESC	A_3D_3	43	#	A_3D_4	53	+	A_3D_5	63	3	A_3D_6	73	;	A_3D_7
004	EOT	A_4D_0	014	FF	A_4D_1	24	DC4	A_4D_2	34	FS	A_4D_3	44	$	A_4D_4	54	,	A_4D_5	64	4	A_4D_6	74	<	A_4D_7
005	ENQ	A_5D_0	015	CR	A_5D_1	25	NAK	A_5D_2	35	GS	A_5D_3	45	%	A_5D_4	55	-	A_5D_5	65	5	A_5D_6	75	=	A_5D_7
006	ACK	A_6D_0	016	SO	A_6D_1	26	SYN	A_6D_2	36	RS	A_6D_3	46	&	A_6D_4	56	.	A_6D_5	66	6	A_6D_6	76	>	A_6D_7
007	BEL	A_7D_0	017	SI	A_7D_1	27	ETB	A_7D_2	37	US	A_7D_3	47	`	A_7D_4	57	/	A_7D_5	67	7	A_7D_6	77	?	A_7D_7
OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM	OCT	CHAR	RAM
100	@	$A_0D'_0$	110	H	$A_0D'_1$	120	P	$A_0D'_2$	130	X	$A_0D'_3$	140	'	$A_0D'_4$	150	h	$A_0D'_5$	160	p	$A_0D'_6$	170	x	$A_0D'_7$
101	A	$A_1D'_0$	111	I	$A_1D'_1$	121	Q	$A_1D'_2$	131	Y	$A_1D'_3$	141	a	$A_1D'_4$	151	i	$A_1D'_5$	161	q	$A_1D'_6$	171	y	$A_1D'_7$
102	B	$A_2D'_0$	112	J	$A_2D'_1$	122	R	$A_2D'_2$	132	Z	$A_2D'_3$	142	b	$A_2D'_4$	152	j	$A_2D'_5$	162	r	$A_2D'_6$	172	z	$A_2D'_7$
103	C	$A_3D'_0$	113	K	$A_3D'_1$	123	S	$A_3D'_2$	133	[	$A_3D'_3$	143	c	$A_3D'_4$	153	k	$A_3D'_5$	163	s	$A_3D'_6$	173	{	$A_3D'_7$
104	D	$A_4D'_0$	114	L	$A_4D'_1$	124	T	$A_4D'_2$	134	\	$A_4D'_3$	144	d	$A_4D'_4$	154	l	$A_4D'_5$	164	t	$A_4D'_6$	174	|	$A_4D'_7$
105	E	$A_5D'_0$	115	M	$A_5D'_1$	125	U	$A_5D'_2$	135	]	$A_5D'_3$	145	e	$A_5D'_4$	155	m	$A_5D'_5$	165	u	$A_5D'_6$	175	}	$A_5D'_7$
106	F	$A_6D'_0$	116	N	$A_6D'_1$	126	V	$A_6D'_2$	136	^	$A_6D'_3$	146	f	$A_6D'_4$	156	n	$A_6D'_5$	166	v	$A_6D'_6$	176	~	$A_6D'_7$
107	G	$A_7D'_0$	117	O	$A_7D'_1$	127	W	$A_7D'_2$	137	_	$A_7D'_3$	147	g	$A_7D'_4$	157	o	$A_7D'_5$	167	w	$A_7D'_6$	177	del	$A_7D'_7$

Note: The first 64 codes list 32 non-printing characters of actions and 32 printing characters, including 10 numbers (0~9). The second 64 codes list the remaining 64 printing characters, including the upper-case and lower-case of letters. OCT stands for octal codes, CHAR stands for characters, RAM stands for Raman codes.

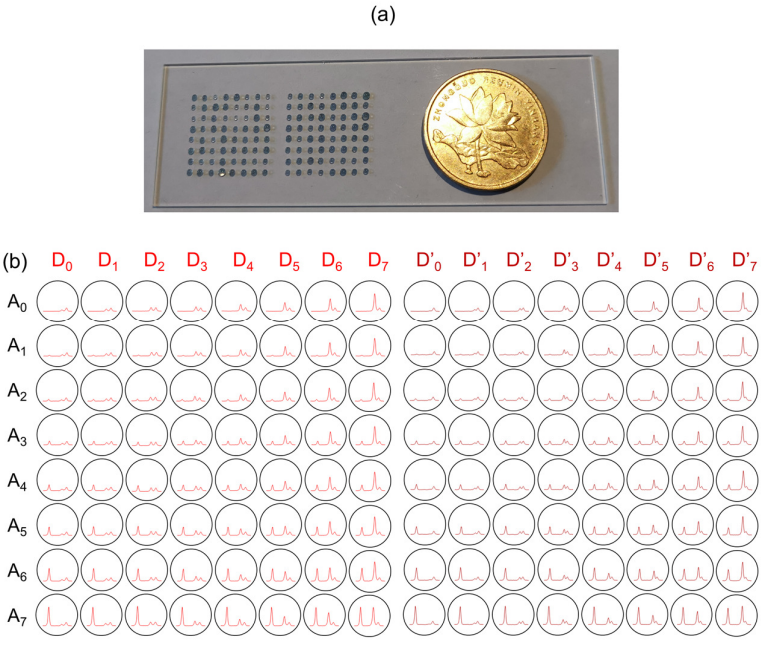


Figure S3. The 128 Raman barcodes expressing the ASCII system. (a) Picture of the 128 encoded solutions spotted on a quartz slide. (b) Individual spectra of the 128 encoded

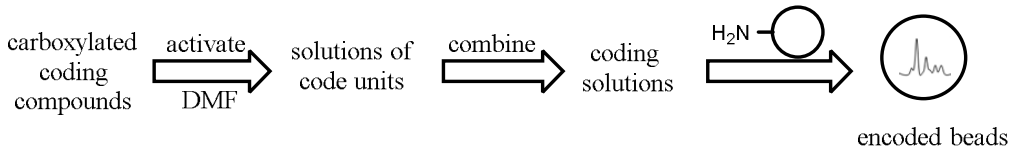
solutions. The left square represents the 64 codes of A_mD_j (m =0~7, j = 0~7, spectra in red). The right square represents the 64 codes of $A_mD'_j$ (m =0~7, j = 0~7, spectra in deep red).

Writing with the Hexadecimal Unicode-Raman Coding System. 0.25 M compound R, 2.6 M compound A, 3.3 M compound A', 0.026 M compound B, 0.033 M compound B', 0.057 M compound C, 0.075 M compound C', 0.10 M compound D and 0.27 M compound D' were prepared as the stock solutions. Blank NMP was used as the coding units A_0, B_0, C_0 and D_0. The stock solutions of A, B, C and D were diluted to seven concentrations by a factor of 1×, 1.5×, 2.25×, 3.38×, 5.06×, 10.13×, 20.25×, respectively to obtain the code units A_1~A_7, B_1~B_7, C_1~C_7 and D_1~D_7. The stock solutions of A', B', C' and D' were diluted to 8 concentrations by a factor of 1×, 1.5×, 2.25×, 3.38×, 5.06×, 6.75×, 10.13×, 20.25× to obtain the code units A_8~A_f, B_8~B_f, C_8~C_f and D_8~D_f.

The coding solutions for each Chinese character in the authors' affiliation "华(534e)中(4e2d)师(5e08)范(8303)大(5927)学(5b66)" were selected and spotted onto the surface (see **Figure 5** in the main text). For example, the code for 华 was made by mixing 5 µL of the stock of R, 5 µL of A_5, 5 µL of B_3, 5 µL of C_4 and 5 µL of D_e. The codes were decoded under a 50× objective using a confocal Raman microscope.

Preparation of Raman Encoded Beads

Preparation of ⓍN Raman Encoded Beads. As shown in Scheme S1, the encoded beads were prepared using the solid-phase peptide synthesis method based on polystyrene resins containing free amino groups. This code-writing method is applicable to the most aminolated polystyrene beads using DVB crosslinking. Applicable bead sizes can vary from 10 µm to 200 µm. Fmoc protected commercial resins such as Rink Amid resin beads were first deprotected by 20% 4-methylpiperidine in DMF. Unprotected commercial resin beads such as TentaGel S-NH_2 or aminomethyl polystyrene resin were used directly. In the experiments below, we used Rink Amide resin beads as an example.



Scheme S1. Preparation of Encoded Beads.

The codes were designed as a geometric series of intensities increasing with a fixed ratio of ~1.5× (Table S2). For example, the intensity of code 1 was about 1/3 of the reference peak, the intensity of code 4 was about the same as the reference peak, and the intensity of code 7 was about 3 times of the reference peak. In this design, there was about 50% intensity increase with increasing codes, which helped to avoid signal cross talking. Experimentally Compound Ⓡ (6.1 mg, 0.0285 mmol)

was dissolved in a mixture including 28.6 μL of 1.0 M Cl-HOBt (0.0286 mmol), 9.3 μL of DIC (0.0602 mmol) and 506 μL of DMF to obtain a solution of 0.052 M activated Ⓡ. Compound Ⓐ (9.6 mg, 0.0658 mmol) was dissolved in a mixture including 66 μL of 1.0 M Cl-HOBt (0.066 mmol), 21.4 μL of DIC (0.138 mmol) and 403 μL of DMF to obtain a solution of 0.132 M activated Ⓐ. The pre-made Ⓡ solution was mixed with the pre-made Ⓐ solution by a predetermined volume ratios of 0, 0.30, 0.45, 0.68, 1.01, 1.52, 2.28 and 3.42, respectively. For example, eight different volumes (0 μL, 30 μL, 45 μL, 68 μL, 101 μL, 152 μL, 228 μL, 342 μL) of Ⓐ were added to 100 μL of the pre-made Ⓡ solution to obtain eight activated coding solutions (Ⓐ+Ⓡ). As long as the pre-determined ratios were maintained, the absolute volumes may vary corresponding to the reaction scales in need.

Table S2. Design of standard codes.

Designed Codes	0	1	2	3	4	5	6	7
Volume ratio of Compound X: R	0	0.3	0.45	0.68	1.01	1.52	2.28	3.42
Expected Relative Raman Intensity	0	0.3	0.45	0.68	1.01	1.52	2.28	3.42

Rink Amide MBHA resin beads (24 mg, loading 0.657 mmol/g, 100-200 mech) were swollen in DMF for 2 h before Fmoc-deprotection with 20% 4-methylpiperidine in DMF twice for 5 min and 15 min each. The beads were washed with 1 mL of DMF for three times, 1 mL of MeOH for three times, and 1 mL of DMF for three times, respectively, and then divided into 8 equal portions (3 mg each). The beads were added to the pre-mixed Ⓐ+Ⓡ coding solutions to start the code-writing process. The coupling reactions were carried out at room temperature for 2 h and monitored by Kaiser Test. After filtration, the beads were washed with 1 mL of DMF for three times, 1 mL of MeOH for three times, and 1 mL of DMF for three times, respectively. A series of 8 codes of $Ⓐ_n$ (n=0~7) were obtained as shown in **Figure 3d**.

Following the same steps described above, 0.052 M activated Ⓡ and 0.0033 M activated Ⓑ were prepared to obtain the second series of coding solutions. 0.13 M activated Ⓡ and 0.010 M activated Ⓒ were prepared to obtain the third series of coding solutions. 0.26 M activated Ⓡ and 0.04 M activated Ⓓ were prepared to obtain the fourth series of activated coding solutions. Then the same procedures were taken for the rest of experiments to obtain $Ⓑ_n$, $Ⓒ_n$ and $Ⓓ_n$ as previously to obtain $Ⓐ_n$ (n=0~7).

Preparation of $Ⓐ_mⒹ_n$ Raman Encoded Beads. 0.052 M activated Ⓡ, 0.132 M activated Ⓐ and 0.010 M activated Ⓓ were prepared to obtain the stock solutions. In order to make 64 coding solutions to produce $Ⓐ_mⒹ_n$ (m = 0~7, n = 0~7), different volume combinations of the stock Ⓐ and Ⓓ were added per 100 μL of the stock Ⓡ (see **Table S3**).

3.0 mg of deprotected Rink Amide resin beads were added to each of the 64 coding solutions.

The coupling reactions were carried out at room temperature for 2 h and the monitored by Kaiser test. After filtration, the beads were washed with 1 mL DMF for three times, 1 mL MeOH for three times, and 1 mL DMF for three times, respectively. A series of 64 codes of $Ⓐ_mⒹ_n$ (m = 0~7, n = 0~7) were obtained.

Table S3. Example of volume combinations to make coding solutions for $Ⓐ_mⒹ_n$.

Code $V_Ⓓ$/μL / $V_Ⓐ$/μL	0	30	45	67.5	101.3	151.9	227.8	341.7
0	$Ⓐ_0Ⓓ_0$	$Ⓐ_0Ⓓ_1$	$Ⓐ_0Ⓓ_2$	$Ⓐ_0Ⓓ_3$	$Ⓐ_0Ⓓ_4$	$Ⓐ_0Ⓓ_5$	$Ⓐ_0Ⓓ_6$	$Ⓐ_0Ⓓ_7$
30	$Ⓐ_1Ⓓ_0$	$Ⓐ_1Ⓓ_1$	$Ⓐ_1Ⓓ_2$	$Ⓐ_1Ⓓ_3$	$Ⓐ_1Ⓓ_4$	$Ⓐ_1Ⓓ_5$	$Ⓐ_1Ⓓ_6$	$Ⓐ_1Ⓓ_7$
45	$Ⓐ_2Ⓓ_0$	$Ⓐ_2Ⓓ_1$	$Ⓐ_2Ⓓ_2$	$Ⓐ_2Ⓓ_3$	$Ⓐ_2Ⓓ_4$	$Ⓐ_2Ⓓ_5$	$Ⓐ_2Ⓓ_6$	$Ⓐ_2Ⓓ_7$
67.5	$Ⓐ_3Ⓓ_0$	$Ⓐ_3Ⓓ_1$	$Ⓐ_3Ⓓ_2$	$Ⓐ_3Ⓓ_3$	$Ⓐ_3Ⓓ_4$	$Ⓐ_3Ⓓ_5$	$Ⓐ_3Ⓓ_6$	$Ⓐ_3Ⓓ_7$
101.3	$Ⓐ_4Ⓓ_0$	$Ⓐ_4Ⓓ_1$	$Ⓐ_4Ⓓ_2$	$Ⓐ_4Ⓓ_3$	$Ⓐ_4Ⓓ_4$	$Ⓐ_4Ⓓ_5$	$Ⓐ_4Ⓓ_6$	$Ⓐ_4Ⓓ_7$
151.9	$Ⓐ_5Ⓓ_0$	$Ⓐ_5Ⓓ_1$	$Ⓐ_5Ⓓ_2$	$Ⓐ_5Ⓓ_3$	$Ⓐ_5Ⓓ_4$	$Ⓐ_5Ⓓ_5$	$Ⓐ_5Ⓓ_6$	$Ⓐ_5Ⓓ_7$
227.8	$Ⓐ_6Ⓓ_0$	$Ⓐ_6Ⓓ_1$	$Ⓐ_6Ⓓ_2$	$Ⓐ_6Ⓓ_3$	$Ⓐ_6Ⓓ_4$	$Ⓐ_6Ⓓ_5$	$Ⓐ_6Ⓓ_6$	$Ⓐ_6Ⓓ_7$
341.7	$Ⓐ_7Ⓓ_0$	$Ⓐ_7Ⓓ_1$	$Ⓐ_7Ⓓ_2$	$Ⓐ_7Ⓓ_3$	$Ⓐ_7Ⓓ_4$	$Ⓐ_7Ⓓ_5$	$Ⓐ_7Ⓓ_6$	$Ⓐ_7Ⓓ_7$

Note: the absolute volumes can be adjusted according to the reaction scales in need.

Preparation of $Ⓐ_3Ⓒ_nⒹ_4$ Raman Encoded Beads. 0.052 M activated Ⓡ, 0.132 M activated Ⓐ, 0.0060 M activated Ⓒ and 0.010 M activated Ⓓ were prepared as the stock solutions. To 100 μL of the stock Ⓡ, a mixture was added, including 68 μL of the stock Ⓐ, 101 μL of the stock Ⓓ, and 8 different volumes of the stock Ⓒ (0 μL, 30 μL, 45 μL, 68 μL, 101 μL, 152 μL, 228 μL, 342 μL). This produced 8 coding solutions. Then the same procedures were taken as described previously to obtain 8 codes of $Ⓐ_3Ⓒ_nⒹ_4$ (n = 0~7).

Preparation of $Ⓐ_3Ⓑ_nⒸ_5Ⓓ_4$ Raman Encoded Beads. 0.052 M activated Ⓡ, 0.132 M activated Ⓐ, 0.0033 M activated Ⓑ, 0.0060 M activated Ⓒ and 0.010 M activated Ⓓ were prepared as the stock solutions. To 100 μL of the stock Ⓡ, a mixture was added including 68 μL of the stock Ⓐ, 152 μL of the stock Ⓒ, 101 μL of the stock Ⓓ, and 8 different volumes of the stock Ⓑ (0 μL, 30 μL, 45 μL, 68 μL, 101 μL, 152 μL, 228 μL, 342 μL). This produced 8 coding solutions. The same procedures were then taken as described previously to obtain 8 codes of $Ⓐ_3Ⓑ_nⒸ_5Ⓓ_4$ (n = 0~7).

Kaiser Test. A few beads were taken from the reaction container and washed 3 times with DMF, 3 times with absolute ethanol, and then transferred to a small tube. 2 drops of 0.001 M potassium cyanide in pyridine, 80% phenol in *n*-butanol solution, and 5% ninhydrin in *n*-butanol solution were added, respectively, and heated in a metal bath at 110℃ for 5 min.

Decoding of Raman Beads. Before measurement, Raman-coded beads were fixed to the

glass slide by vacuum grease. Then the glass slide was placed under a 50× objective. The light beam was focused at the center of the beads with light intensity of 15 mW/μm^2. For each bead, 10 spectral frames were collected for 2 s exposure time each. Five beads were measured for each batch of Ⓧ$_n$ (X = A, B, C, D, n = 0~7). One bead was measured for each batch of Ⓐ$_m$ Ⓓ$_n$, Ⓐ$_3$Ⓒ$_n$Ⓓ$_4$, and Ⓐ$_3$Ⓑ$_n$Ⓒ$_5$Ⓓ$_4$ (m, n = 0~7).

Signal Stability of Encoded Beads. After the eight Raman codes of Ⓐ$_3$Ⓑ$_n$Ⓒ$_5$Ⓓ$_4$ (n = 0~7) were prepared, the same batches were measured within one day and after 150 days. The consistency between these two types of spectra verified that, the encoded beads can be stably stored in the ambient environment (room light and temperature) without degradation of signals.

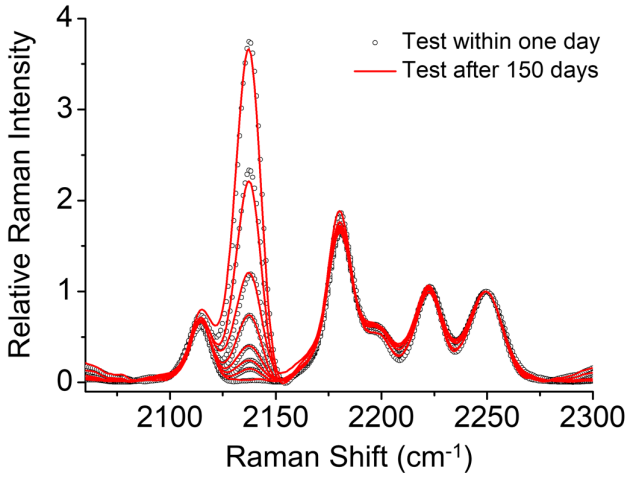


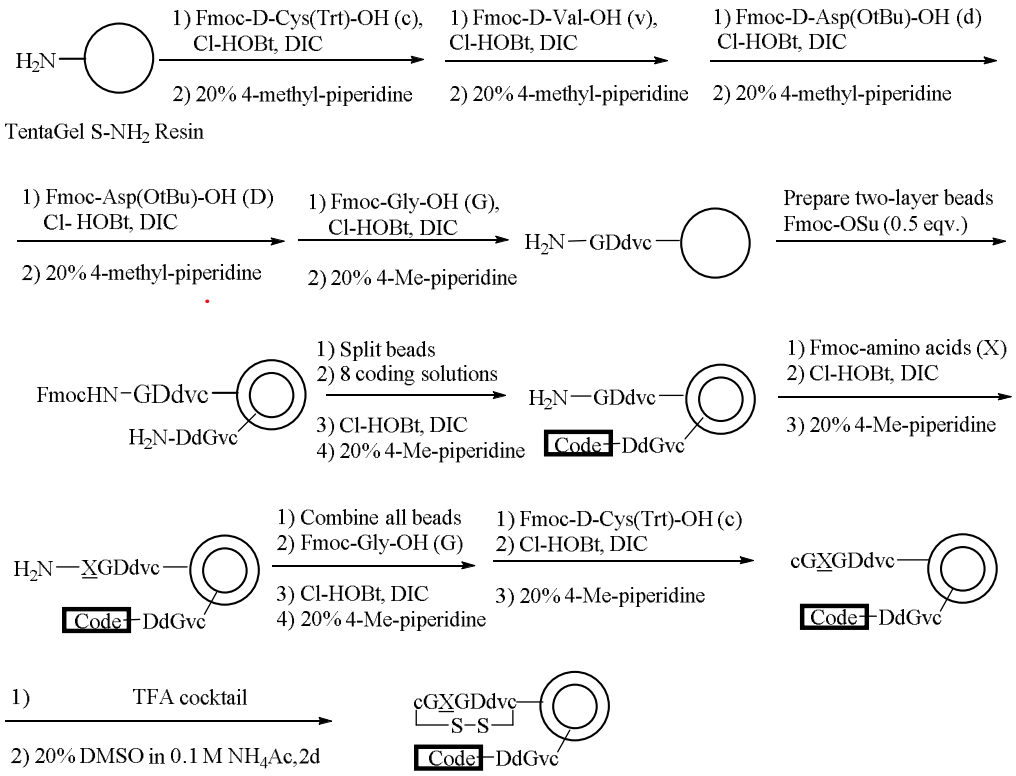
Figure S4. Raman Spectra of Ⓐ$_3$Ⓑ$_n$Ⓒ$_5$Ⓓ$_4$ (n = 0~7) measured within one day and 150 days after fresh preparation.

Synthesis and Screening of the Encoded Peptide Library

Solid-Phase Synthesis of the Encoded Peptide Library. The synthetic approach of the OBOC peptide library coding is shown in **Scheme S2**.

TentaGel S-NH_2 beads (300 mg, loading 0.26 mmol/g) were swollen in DMF (2 mL) for 4 h. Fmoc-D-cys(Trt)-OH (0.137 g, 0.234 mmol) was dissolved in a solution of Cl-HOBt (0.040 g, 0.234 mmol) and DIC (145 μL, 0.468 mmol) in DMF, and was then added into the beads. The coupling was carried out at room temperature for 2 h. After filtration, the beads were washed with 2 mL DMF for three times, 2 mL MeOH for three times, and 2 mL DMF for 3 times, respectively. The Fmoc group was removed with 20% 4-methylpiperidine twice (5 min and 15 min each) at room temperature. The beads were then subjected to additional coupling and deprotection cycles with Fmoc-D-Val-OH, Fmoc-D-Asp(O*t*Bu)-OH, Fmoc-L-Asp(O*t*Bu)-OH and Fmoc-L-Gly-OH in the same way as described above. After the removal of Fmoc, the

beads were washed with DMF 2 mL DMF for three times, 2 mL MeOH for three times, and 2 mL DMF for 3 times, and 2 mL DCM for 6 times, respectively. [1]



Scheme S2. Raman Encoding of the Focused OBOC Cyclic Peptide Library.

Two-layer beads were then prepared using a bi-phasic solvent approach.[2] The beads with a free amino group at the N-terminus were dried in vacuum completely and then swollen in water for 24 h. Water was removed by filtration, and a mixture including 0.013 g 9-fluorenylmethyloxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu) (0.039 mmol), 32 μL of DIEA (0.189 mmol) and 8 mL of DCM/diethyl ether (volume ratio = 55% : 45%) was added to the beads and the mixture was rigorously shaken for 30 min. The beads were then filtered and washed three times with DCM/diethyl ether and six times with DMF to remove water from the beads. Then the inner layer of the beads was used to write the Raman codes.

The beads were divided into 8 equal portions. Then 8 Raman codes were coupled to the interior of the beads using the compounds Ⓓ and Ⓡ as described previously. After Fmoc removal with 20% 4-methylpiperidine at rt, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Met-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Arg(Bpf)-OH, Fmoc-Phe-OH, Fmoc-D-Phe-OH and Fmoc-D-Val-OH was coupled to the beads in DMF with DIC/Cl-HOBt activation. After this diversity-generating step, 8 aliquots were pooled together for the next synthetic steps.

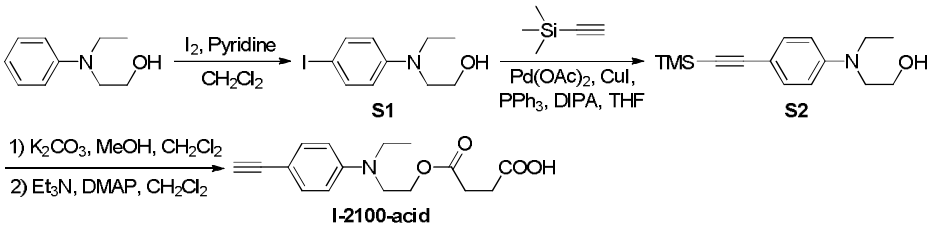
Fmoc-Gly-OH and Fmoc-D-Cys(Trt)-OH was then coupled to the beads sequentially. After removal of Fmoc, the beads were washed with 2 mL DMF for three times, 2 mL of MeOH for three times, 2 mL of DCM for three times, and then dried in vacuum for 1 h. Side-chain deprotection was achieved using a mixture of 82.5% TFA/5% phenol/5% thioanisole/5% water/2.5% TIS (v/v). The cleavage reaction was conducted at room temperature for 2 h. After neutralization with 10% DIEA/DMF (twice), the beads were washed sequentially with 2 mL of DMF for three times, 2 mL of MeOH for three times, 2 mL of DCM for three times, 2 mL of DMF for three times, 2 mL of DMF/water (60%/30%) for 3 times, 2 mL of water for three times, and 2 mL of PBS for ten times, respectively. Then the beads were transferred to a 10 mL bottle, into which 10 mL mixture of water, acetic acid and DMSO (volume ratio = 75 : 5 : 20, pH = 6) was added. The beads were shaken for two days until the Ellman test was negative. After filtration, the beads were washed with H_2O and PBS. Finally, the bead library was stored in PBS at the concentration of 20 mg/mL for the subsequent screening process.

Cell Binding Assay. U-87MG cells were trypsinized with 0.05% trypsin-EDTA from the bottom of a T75 flask and neutralized with culture medium. Overgrown floating cells were collected, spun down, and resuspended in 4 mL of culture medium. 2 mL of suspension cells in culture medium was placed into a 35 mm Petri dish. The pre-stored beads were added and incubated with U-87MG cells at 37℃ with gentle shaking (40 rpm) in a humidified incubator for 2 h. After incubation, the sample was placed on a clean quartz container (10 mm diameter, 1 mm deep). The cell binding was observed under a 10× objective. The Raman spectra of beads were collected under a 50× long distance objective.

Synthesis of Raman Coding Compounds

Band I Compounds (Substituted Phenylacetylene).

Ⅰ-2100-acid[3]



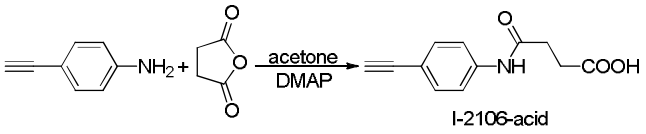
A solution of N-ethyl-N-hydroxyethylaniline (330 mg, 2.0 mmol) and pyridine (325 μL, 4.0 mmol) in CH_2Cl_2 was cooled in an ice bath until the internal temperature reached 5 °C, (325 μL, 4.0 mmol) of I_2 was added portionwise over 30 min and stirred at room temperature for 1 h. The reaction mixture was washed with H_2O three times, then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **S1** (410 mg, 70%) as a pale green liquid.

To a solution of **S1** (291 mg, 1.0 mmol) and trimethylsilylacetylene (170 μL, 1.2 mmol) in THF (10 mL) was added diisopropylamine (495 μL, 3.0 mmol). The mixture was stirred while N_2 was slowly bubbled through the mixture for 0.5 h. Triphenylphosphine (34 mg, 0.10 mmol) and CuI (12 mg, 0.063 mmol) were added to the mixture and allowed to dissolve completely for 10 min. $Pb(OAc)_2$ (1.0 mg, 0.0045 mmol) was then added, and the mixture was gently refluxed for 1 h. The mixture was cooled to rt and subjected to chromatography to give **S2** (160 mg, 61%) as a light yellow solid.

To a solution of **S2** (110 mg, 0.42 mmol) in CH_2Cl_2 : MeOH (1 : 1, 10 mL) was added K_2CO_3 (290 mg, 2.1 mmol). The reaction mixture was filtering at reduced pressure after stirring at rt for 2 h. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, then dried over Na_2SO_4. After filtration, the solution was used directly for next step.

To the above solution was added succinic anhydride (50 mg, 0.50 mmol), Et_3N (175 μL, 1.26 mmol) and DMAP (26 mg, 0.21 mmol). After the mixture was stirred at 30 ℃ overnight, H_2O was added to the reaction and the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅰ-2100-acid** (80 mg, 66%) as a pale yellow solid. ^{1}HNMR (400 MHz, DMSO) δ 11.69 (s, 1H), 7.43 (d, *J* = 8.8 Hz, 2H), 6.59 (d, *J* = 8.8 Hz, 2H), 4.16 (t, *J* = 5.8 Hz, 2H), 3.52 (t, *J* = 5.8 Hz, 2H), 3.37 (dd, *J* = 13.8, 6.8 Hz, 2H), 2.57 – 2.52 (m, 5H), 1.08 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.84, 172.65, 147.36, 137.79, 137.79, 130.90, 114.83, 114.83, 110.87, 76.84, 61.82, 48.55, 44.95, 29.14, 29.03, 12.12. ESI-MS: calcd for $C_{16}H_{20}NO_4^+$ $[M+H]^+$ 290.13868, found 290.16635.

I-2106 / Ⅰ-2106-acid

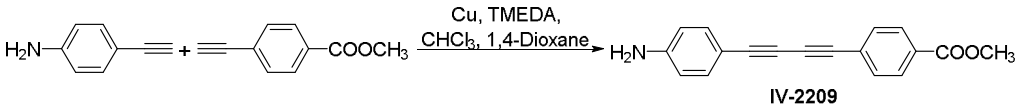


To a solution of 4-ethynylaniline (585 mg, 5.0 mmol) and succinic anhydride (600 mg, 6.0 mmol) in acetone (40 mL) was added 4-dimethylaminopyridine (610 mg, 5.0 mmol). The reaction mixture was stirred at 60 ℃ for 2 days. H_2O was added to the mixture and the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅰ-2160-acid** (565 mg, 52%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.18 (s, 1H), 10.18 (s, 1H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 4.10 (s, 1H), 2.60 (d, *J* = 5.3 Hz, 2H), 2.55 (d, 2H).^{13}C NMR (101 MHz, DMSO) δ 174.26, 170.85, 140.28, 132.82, 132.82, 119.13, 119.13, 116.23, 84.06, 80.18, 31.54, 29.12.

ESI-MS: calcd for $C_{12}H_{10}NO_3^-$ $[M-H]^-$ 216.06662, found 216.06620.

Band IV and Band R Compounds (Di-Alkynes)

Ⅳ-2209

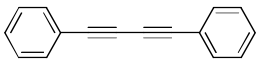


IV-2209

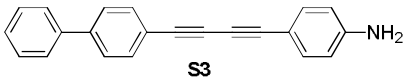
To a solution of methyl 4-ethynylbenzoate (800 mg, 5.0 mmol) and 4-ethynylaniline (877 mg, 7.5 mmol) in $CHCl_3$-1,4-dioxane (3 : 1, 16 mL) were added Cu powder (32 mg, 0.50 mmol) and TMEDA (226 μL, 1.5 mmol). After the mixture was stirred at 50 ℃ overnight, enough aqueous NH_4Cl was added. The mixture was extracted three times with EtOAc. The organic phase washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **IV-2209** (725 mg, 53%) as a light yellow solid. ^{1}HNMR (400 MHz, DMSO) δ 7.99 (d, *J* = 8.2 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 6.58 (d, *J* = 8.4 Hz, 2H), 5.92 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.97, 151.43, 134.54, 134.54, 132.80, 132.80, 130.10, 129.86, 129.86, 126.49, 114.02, 114.02, 105.39, 86.78, 80.27, 78.04, 71.52, 52.85.ESI-MS: calcd for $C_{18}H_{14}NO_2^+$ $[M+H]^+$ 276.10191, found 276.10193.

The following compounds were prepared using the same coupling procedures as **Ⅳ-2209**.

Ⅳ-2223


IV-2223

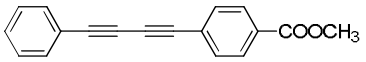
White soild. ^{1}HNMR (400 MHz, DMSO) δ 7.69 – 7.60 (d, 4H), 7.55 – 7.50 (t, 2H), 7.47 (t, *J* = 11.3, 4.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 132.87, 132.87, 132.87, 132.87, 130.49, 130.49, 129.39, 129.39, 129.39, 129.39, 120.85, 120.85, 82.29, 82.29, 73.94, 73.94.ESI-MS: calcd for $C_{16}H_{11}^+$ $[M+H]^+$ 203.08553, found 203.08832.



S3

Light yellow soild.^{1}H NMR (400 MHz, DMSO) δ 7.74 (d, *J* = 7.0 Hz, 4H), 7.67 (d, *J* = 7.9 Hz, 2H), 7.52 (t, *J* = 6.9 Hz, 2H), 7.43 (t, *J* = 6.4 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 2H), 6.59 (d, *J* = 8.3 Hz, 2H), 5.88 (s, 2H).^{13}C NMR (101 MHz, DMSO) δ 151.19, 141.26, 139.42, 134.39, 134.39, 133.17, 133.17, 129.54, 129.54, 128.53, 127.46, 127.46, 127.18, 127.18, 120.62,

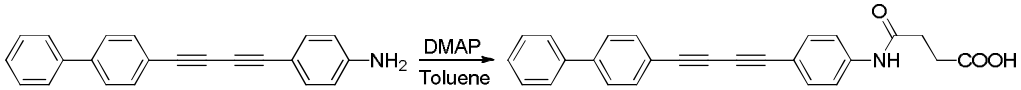
114.05, 114.05, 105.87, 85.38, 81.12, 75.89, 71.80.



S4

White soild. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, *J*= 4.0 Hz, 2 H), 7.57-7.50 (m, 4 H), 7.39-7.30 (m, 3 H) 3.90 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.2, 132.5, 132.3, 130.2, 129.5, 129.4, 128.4, 126.4, 121.3, 82.9, 80.4, 76.6, 73.5, 52.3; HRMS calcd for $C_{18}H_{12}O_2$: 260.0837, found: 260.0840.

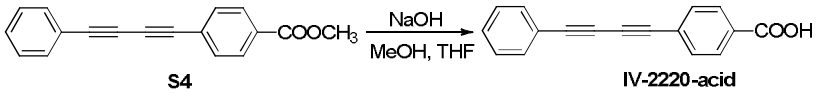
Ⅳ-2215-acid



S3 IV-2215-acid

To a solution of **S3** (117 mg, 0.40 mmol) and succinic anhydride (48 mg, 0.48 mmol) in acetone (20 mL) was added 4-dimethylaminopyridine (49 mg, 0.40 mmol). The reaction mixture was stirred at 60 ℃ for 3 days. H_2O was added to the mixture and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅳ-2215-acid** (50 mg, 32%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.29 (s, 1H), 10.30 (s, 1H), 7.81 – 7.66 (m, 8H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.52 (t, *J* = 7.4 Hz, 2H), 7.44 (t, *J* = 7.1 Hz, 1H), 2.62 (d, *J* = 5.7 Hz, 2H), 2.59 – 2.55 (d, 2H).^{13}C NMR (101 MHz, DMSO) δ 174.27, 171.06, 141.73, 141.26, 139.35, 133.78, 133.78, 133.42, 133.42, 129.55, 129.55, 128.63, 127.53, 127.53, 127.23, 127.23, 120.01, 119.24, 119.24, 114.62, 83.08, 81.92, 75.00, 73.34, 31.63, 29.13. ESI-MS: calcd for $C_{26}H_{18}NO_3^-$ $[M-H]^-$ 392.12922, found 392.12866.

Ⅳ-2220-acid

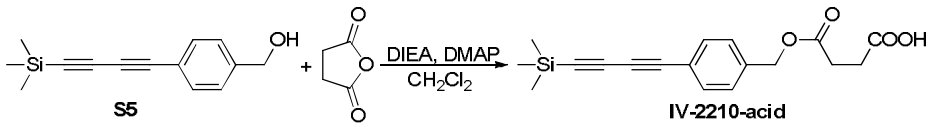


S4 IV-2220-acid

To a solution of **S4** (728 mg, 2.8 mmol) in MeOH-THF (1 : 1, 20 mL) was added aqueous NaOH (560 mg, 14 mmol), the reaction mixture was stirred 3 h at rt. Then, the mixture was concentrated at reduced pressure, CH_2Cl_2 and H_2O was added, followed by addition of aqueous HCl (1 M) to adjust pH to 5. The mixture was extracted with CH_2Cl_2, the organic phase was washed with H_2O and brine, dried over Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅳ-2220-acid** as a white solid (650 mg, 94%). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.98 (d, *J* = 7.9 Hz, 2H), 7.73

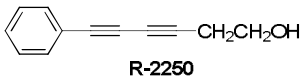
(d, *J* = 7.8 Hz, 2H), 7.66–7.62 (m, 2H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 166.96, 133.07, 132.98, 132.03, 130.74, 130.04, 129.44, 125.17, 120.59, 83.56, 81.34, 76.28, 73.65. HRMS (ESI) m/z for $[M-H]^-$, $C_{17}H_9O_2^-$: calcd, 245.0608; found, 245.0603.[5]

Ⅳ-2210-acid



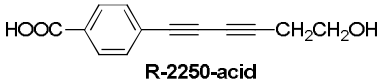
A solution of **S5** (43 mg, 0.15 mmol) and succinic anhydride (45 mg, 0.45 mmol) was added N,N-diisopropylethylamine (74 μL, 0.45 mmol) and 4-dimethylaminopyridine (9.2 mg, 0.075 mmol). The reaction mixture was stirred at 40 ℃ overnight. H_2O was added to the mixture and the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **IV-2210-acid** (43 mg, 87%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.30 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.43 (d, *J* = 8.1 Hz, 2H), 5.16 (s, 2H), 2.62 (t, *J* = 6.2 Hz, 2H), 2.54 (t, 2H), 0.25 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 173.87, 172.45, 138.93, 133.14, 133.14, 128.40, 128.40, 119.83, 91.88, 88.23, 77.23, 74.36, 65.27, 29.14, 29.09, -0.16, -0.16, -0.16. ESI-MS: calcd for $C_{18}H_{19}O_4Si^-$ $[M-H]^-$ 327.10581, found 327.10775.

R-2250



Following the same procedure of **IV-2210**， **R-2250** was obtained as a colorless liquid. ^{1}HNMR (400 MHz, DMSO) δ 7.56 (d, 2H), 7.52 – 7.39 (m, 3H), 5.02 (t, *J* = 4.9 Hz, 1H), 3.58 (m, 2H), 2.57 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 132.80, 132.80, 130.03, 129.29, 129.29, 121.25, 84.62, 75.10, 74.75, 65.74, 59.65, 23.82.ESI-MS: calcd for $C_{12}H_{11}O^+$ $[M+H]^+$ 171.08044, found 171.09798.

R-2250-acid

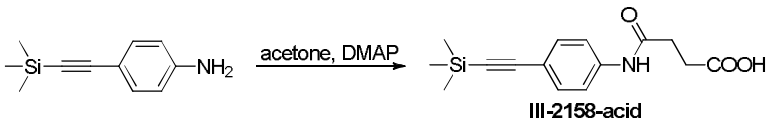


Following the same procedure of compound **IV-2220-Acid**， this compound was obtained as a light yellow solid. ^{1}HNMR (400 MHz, DMSO) δ 12.99 (s, 1H), 7.96 (d, *J* = 8.3 Hz, 2H),

7.67 (d, *J* = 8.3 Hz, 2H), 5.04 (s, 1H), 3.59 (t, *J* = 6.5 Hz, 2H), 2.59 (t, *J* = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 167.01, 132.99, 132.99, 131.66, 129.97, 129.97, 125.60, 86.24, 77.17, 74.21, 65.50, 59.55, 23.86.ESI-MS: calcd for $C_{13}H_9O_3^-$ $[M-H]^-$ 213.05572, found 213.05529.

Band III Compounds (TMS Substituted Phenylacetylenes and Tri-Alkynes)

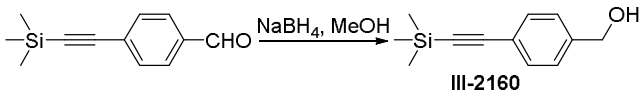
Ⅲ-2158-acid



III-2158-acid

To a solution of 4-(2-(trimethylsilyl)ethynyl)aniline (567 mg, 3.0 mmol) and succinic anhydride (360 mg, 3.6 mmol) in acetone (20 mL) was added 4-dimethylaminopyridine (366 mg, 3.0 mmol). The reaction mixture was stirred at 60 ℃ overnight. H_2O was added to the mixture and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅲ-2158-acid** (580 mg, 67%) as a light yellow solid.

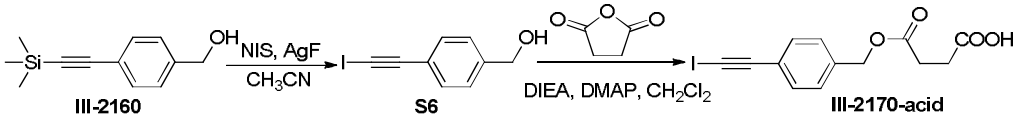
Ⅲ-2160



III-2160

A solution of 4-(trimethylsilyl)ethynylbenzaldehyde (1.01 g, 5.0 mmol) in MeOH was cooled in an ice bath, then sodium borohydride (380 mg, 10 mmol) in cooled MeOH was added portion wise and stirred at room temperature for 0.5 h. H_2O was added to the reaction, followed by aqueous HCl (1 M) addition to make pH 5. The mixture was extracted with CH_2Cl_2 , The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and the solvent was rotary evaporated, leaving a yellow oil that crystallized into a low-melting solid 4-(trimethylsilylethynyl)benzyl alcohol (**Ⅲ-2160**) (989 mg, 97%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.1 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 4.67 (d, *J* = 5.7 Hz, 2H), 1.89 (t, *J* = 5.7 Hz, 1H), 0.25 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.17, 132.12, 126.61, 122.28, 104.87, 94.19, 64.87, - 0.03. ESI-MS: calcd for $C_{12}H_{16}OSiNa^+$, $[M + Na]^+$ 227.0868, found 227.0885.

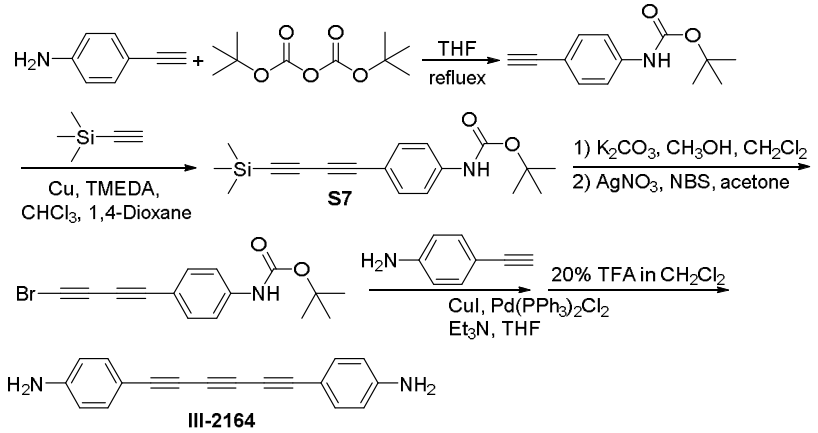
Ⅲ-2170-acid



To a solution of 4-(trimethylsilylethynyl)benzyl (**Ⅲ-2160**) (408 mg, 2.0 mmol) in CH_3CN (10 mL) was added N-iodosuccinimide (540 mg, 2.4 mmol), AgF (254 mg, 2.0 mmol) and H_2O (72 μL, 4.0 mmol). The reaction was in the dark place and stirred at rt overnight. Then, the mixture was filtered under reduced pressure, and the filtrate was poured into H_2O. The product mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4, The solvent was concentrated under reduced pressure and the residue was purified by column chromatography to afford the (4-ethynylphenyl)methanol iodide product **S6** (480 mg, 93%) as a white solid.

A solution of **S6** (387 mg, 1.5 mmol) and succinic anhydride (450 mg, 4.5 mmol) was added N,N-diisopropylethylamine (745 μL, 4.5 mmol) and 4-dimethylaminopyridine (92 mg, 0.75 mmol). The reaction mixture was stirred at 40 ℃ overnight. H_2O was added to the mixture and the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅲ-2170-acid** (340 mg, 63%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.22 (s, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 5.13 (s, 2H), 2.61 (d, 2H), 2.51 (d, 2H).^{13}C NMR (101 MHz, DMSO) δ 173.84, 172.46, 137.44, 132.44, 132.44, 128.26, 128.26, 122.93, 92.77, 65.39, 29.15, 29.10, 18.84. ESI-MS: calcd for $C_{13}H_{10}IO_4^-$ $[M-H]^-$ 356.96293, found 356.96222.

Ⅲ-2164



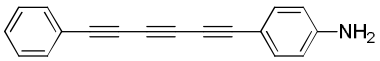
To a solution of 4-ethynylaniline (702.9 mg, 6.0 mmol) in THF (5 mL) was added

di-*tert*-butyl dicarbonate (3.92 g, 18 mmol). After the mixture was stirred at 70℃ overnight, H_2O was added to the reaction and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give N-Boc-4-ethynylaniline compound (1.24 g, 95%) as a white solid.

A solution of N-Boc-4-ethynylaniline (1.24 g, 5.7 mmol) and trimethylsilylacetylene (1.2 mL, 8.55 mmol) in $CHCl_3$-1,4-dioxane (3 : 1, 8 mL) were added Cu (39 mg, 0.60 mmol) and TMEDA (272 μL, 1.8 mmol). After the mixture was stirred at 50 ℃ overnight, aqueous NH_4Cl was added. The mixture was extracted three times with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **S7** (1.30 g, 73%) as a light yellow solid.

To a solution of **S7** (313 mg, 1.0 mmol) in CH_2Cl_2 : MeOH (1 : 1, 10 mL) was added K_2CO_3 (690 mg, 5.0 mmol). The reaction mixture was filtering at reduced pressure after stirring at rt for 2 h. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, then dried over Na_2SO_4. The solvent was concentrated under reduced pressure. Then acetone (10 mL), NBS (196 mmol, 1.1 mmol) and $AgNO_3$ were added. The reaction was stirred at dark place for 2 h. Then, the mixture was filtered under reduced pressure, and the filtrate was poured into H_2O. The product mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over Na_2SO_4, solvent was concentrated under reduced pressure. Then THF (10 mL), methyl 4-Ethynylbenzoate (117 mg, 1.0 mmol), CuI (19 mg, 0.10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol) and Et_3N (166 μL, 1.2 mmol) were added. After the mixture was stirred at rt for 1 h, aqueous NH_4Cl was added. The mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography. 20% TFA in CH_2Cl_2 was added. The mixture was stirred at rt for 1 h. Then, the mixture was concentrated at reduced pressure, EtOAc was added, followed by addition of Et_3N to adjust pH to 7. The mixture was extracted with EtOAc, the organic phase was washed with H_2O and brine, dried over Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅲ-2164** (144 mg, 56%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 7.29 (d, J = 8.5 Hz, 4H), 6.56 (d, J = 8.5 Hz, 4H), 5.95 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ 151.48, 151.48, 134.97, 134.97, 134.97, 134.97, 114.05, 114.05, 114.05, 114.05, 104.92, 104.92, 82.28, 82.28, 72.66, 72.66, 67.27, 67.27. ESI-MS: calcd for $C_{18}H_{13}N_2^+$ $[M+H]^+$ 257.10732, found 257.10774.

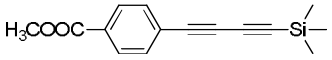
Ⅲ-2168



III-2168

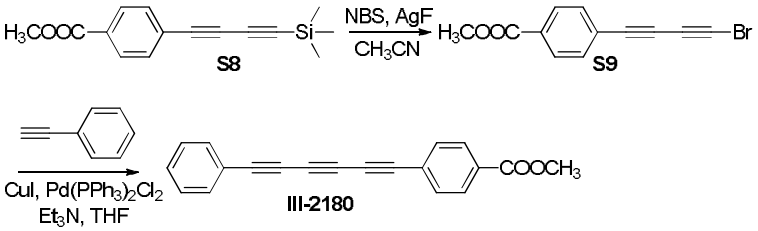
Following the same procedure of compound **III-2164**，compound **III-2168** was obtained as a light yellow solid. ^{1}HNMR (400 MHz, DMSO) δ 7.65 (d, *J* = 7.4 Hz, 2H), 7.57 – 7.50 (m, 1H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 6.57 (t, *J* = 8.5 Hz, 2H), 6.03 (br, 2H). ^{13}C NMR (101 MHz, DMSO) δ 151.76, 135.25, 135.25, 133.29, 133.29, 130.77, 129.43, 129.43, 120.33, 114.10, 114.10, 104.27, 82.95, 79.45, 74.62, 72.31, 68.04, 66.16.ESI-MS: calcd for $C_{18}H_{12}N^{+}$ $[M+H]^{+}$ 242.09643, found 242.09714.

Ⅲ-2180



S8

Following the same procedure of compound **IV-2210**，compound **S8** was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 3.89 (s, 3H), 0.25 (s, 9H).^{13}C NMR (101 MHz, DMSO) δ 165.86, 133.42, 133.42, 130.87, 129.86, 129.86, 125.18, 93.50, 87.77, 76.67, 76.24, 52.90, -0.24, -0.24, -0.24.

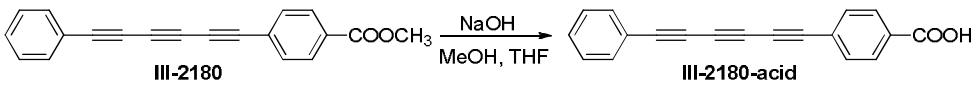


To a solution of **S8** (896 mg, 3.5 mmol) in CH_3CN (20 mL) were added N-bromosuccinimide (748 mg, 4.2 mmol) and AgF (445 mg, 3.5 mmol). The reaction was stirred at rt overnight in dark place. Then, the mixture was filtered under reduced pressure, and the filtrate was poured into H_2O. The product mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by column chromatography to afford the product **S9** (850 mg, 92%) as a light yellow solid.

To a solution of **S9** (263 mg, 1.0 mmol) and phenylacetylene (165 μL, 1.5 mmol) in THF (10 mL) were added CuI (9.5 mg, 0.050 mmol), $Pd(PPh_3)_2Cl_2$ (35 mg, 0.050 mmol) and Et_3N (277 μL, 2.0 mmol). After the mixture was stirred at rt for 1 h, aqueous NH_4Cl was added. The mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine,

dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅲ-2180** (190 mg, 67%) as a white solid. ^{1}HNMR (400 MHz, DMSO) δ 8.02 (d, *J* = 8.3 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.71 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 2H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.83, 133.86, 133.86, 133.59, 133.59, 131.34, 131.26, 129.93, 129.93, 129.51, 129.51, 124.57, 119.61, 80.53, 78.51, 76.47, 73.84, 67.88, 66.18, 52.97. ESI-MS: calcd for $C_{20}H_{13}O_2^+$ $[M+H]^+$ 285.09101, found 285.08861.

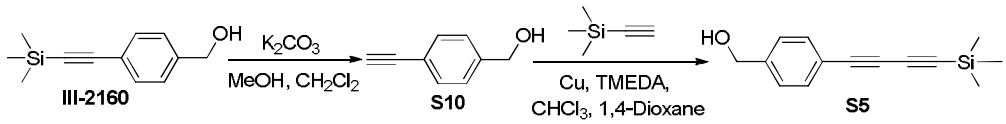
Ⅲ-2180-acid



To a solution of **Ⅲ-2180** (142 mg, 0.50 mmol) in MeOH-THF (1 : 1, 10 mL) was added aqueous NaOH (100 mg, 2.5 mmol), The reaction mixture was stirred 4 h at rt. Then, the mixture was concentrated at reduced pressure, EtOAc and H_2O was added, followed by addition of aqueous HCl (1 M) to adjust pH to 5. The mixture was extracted with EtOAc and the organic phase was washed with H_2O and brine, dried over Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅲ-2180-acid** (125 mg, 93%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 13.31 (s, 1H), 8.00 (d, *J* = 6.2 Hz, 2H), 7.80 (d, *J* = 6.2 Hz, 2H), 7.70 (d, *J* = 6.6 Hz, 2H), 7.56 (d, *J* = 5.9 Hz, 1H), 7.50 (d, *J* = 6.6 Hz, 2H).^{13}C NMR (101 MHz, DMSO) δ 166.90, 133.70, 133.70, 133.57, 133.57, 132.69, 131.30, 130.05, 130.05, 129.50, 129.50, 124.06, 119.65, 80.43, 78.74, 76.17, 73.87, 67.72, 66.26. ESI-MS: calcd for $C_{19}H_9O_2^-$ $[M-H]^-$ 269.06080, found 269.05990.

Band II Compounds (Tetra-Alkynes)

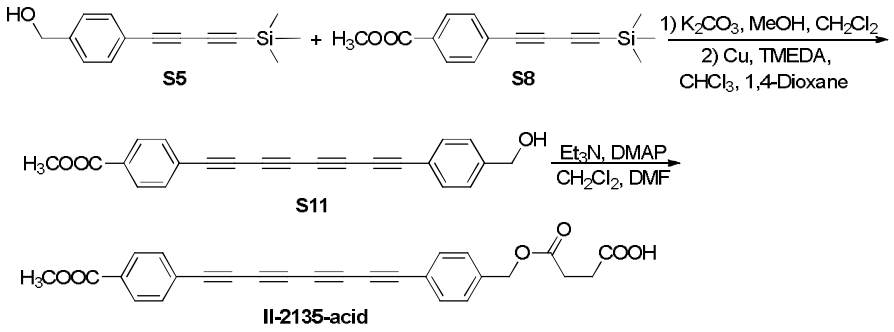
Ⅱ-2135-acid



To a solution of **Ⅲ-2160** (816 mg, 4.0 mmol) in CH_2Cl_2-MeOH (1 : 1, 15 mL) was added K_2CO_3 (2.76 g, 20 mmol). The mixture was stirred at room temperature for 2 h and filtered at reduced pressure. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and the solvent was rotary evaporated to give compound **S10** (510 mg, 97%) as a white solid.

To a solution of **S10** (396 mg, 3.0 mmol) and trimethylsilylacetylene (635 μL, 4.5 mmol) in $CHCl_3$-1,4-dioxane (3 : 1, 8 mL) were added Cu powder (19 mg, 0.30 mmol) and TMEDA

(136 μL, 0.9 mmol). After the mixture was stirred at 50 ℃ overnight, enough aqueous NH_4Cl was added. The mixture was extracted three times with EtOAc. The organic phase washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **S5** (510 mg, 75%) as a white solid. ^{1}H NMR (400 MHz; CDCl3): δ 7.47 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 4.67 (s, 2H), 1.95 (br, 1H), 0.23 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 142.3, 132.9, 126.9, 120.6, 90.8, 87.9, 76.7, 74.3, 64.8, -0.3; HRMS: calcd. for $C_{14}H_{15}Si^+$ $[M\text{-}OH]^+$ 211.0943, found 211.1019.



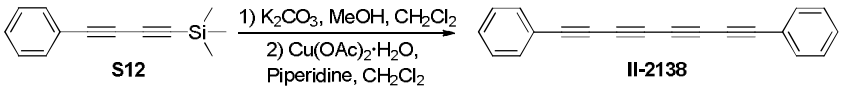
To a solution of **S5** (568 mg, 2.5 mmol) and **S8** (512 mg, 2.0 mmol) in CH_2Cl_2-MeOH (1 : 1, 20 mL) was added K_2CO_3 (2.76 g, 20 mmol). The mixture was stirred at room temperature for 2 h and filtered at reduced pressure. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was used directly for next step.

A solution of above mixture in $CHCl_3$-1,4-dioxane (3 :1 , 24 mL) were added Cu powder (13 mg, 0.20 mmol) and TMEDA (91 μL, 0.60 mmol). After the mixture was stirred at 50 ℃ for 2 days, concentrated at reduced pressure, and then aqueous NH_4Cl was added. The mixture was extracted three times with EtOAc. The combined organic phase was washed with H_2O and brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was subjected to chromatography to give compound **S11** (245 mg, 36%) as a light yellow solid.

To a solution of **S11** (50 mg, 0.15 mmol) and succinic anhydride (45 mg, 0.45 mmol) in CH_2Cl_2-DMF (10 : 1, total 11 mL) were added Et_3N (83 μL, 0.60 mmol) and 4-dimethylaminopyridine (9 mg, 0.074 mmol). The mixture was stirred at 40 ℃ for 6 h and concentrated at reduced pressure. H_2O was added and the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅱ-2135-acid** (30 mg, 46%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.27 (s, 1H), 8.00 (t, 2H), 7.83 (d, *J* = 4.3 Hz, 2H), 7.70 (d, *J* = 5.2 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 5.18

(s, 2H), 3.89 (s, 3H), 2.92 (s, 1H), 2.76 (s, 1H), 2.62 (d, J = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 173.87, 172.45, 165.77, 140.04, 134.16, 134.16, 133.97, 133.97, 131.53, 129.94, 129.94, 128.42, 128.42, 124.03, 118.51, 79.37, 77.90, 76.25, 73.98, 68.19, 66.92, 65.20, 64.78, 63.74, 52.99, 29.12, 29.10. ESI-MS: calcd for $C_{27}H_{17}O_6^-$ $[M-H]^-$ 437.10306, found 437.10037.

Ⅱ-2138

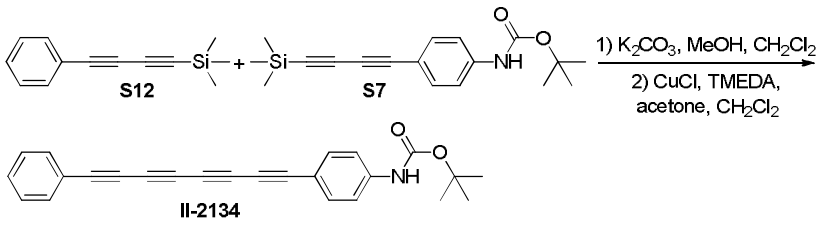


Compound **S12** was obtained as a white solid by following the same procedure as **S5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.48 (m, 2H), 7.40-7.30 (m, 3H), 0.25 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 132.8, 129.4, 128.5, 121.6, 90.8, 88.0, 76.9, 74.3, -0.2; HRMS: calcd. for $C_{13}H_{14}Si^+$ $[M]^+$ 198.0865, found 198.0873.

To a solution of **S12** (693 mg, 3.5 mmol) in CH_2Cl_2-MeOH (1 : 1, 20 mL) was added K_2CO_3 (2.415 g, 17.5 mmol). The reaction mixture was stirred at rt for 2 h and filtered at reduced pressure. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4. The obtained filtrate solution was used directly for next step.

To the above solution were added $Cu(OAc)_2·H_2O$ (35 mg, 0.175 mmol) and piperidine (520 μL, 5.25 mmol). After the mixture was stirred at rt overnight, aqueous NH_4Cl was added to quench the reaction. The mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅱ-2138** (150 mg, 34%) as a yellow solid. ^{1}HNMR (400 MHz, DMSO) δ 7.71 (d, 4H), 7.58 (t, J = 7.5 Hz, 2H), 7.49 (t, J = 7.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 133.83, 133.83, 133.83, 133.83, 131.55, 131.55, 129.54, 129.54, 129.54, 129.54, 119.25, 119.25, 79.21, 79.21, 73.88, 73.88, 67.02, 67.02, 64.15, 64.15. ESI-MS: calcd for $C_{20}H_{10}$ $[M]^+$ 250.0777, found 250.0784.

Ⅱ-2134



To mixture of **S12** (148 mg, 0.75 mmol) and **S7** (157 mg, 0.50 mmol) in CH_2Cl_2-MeOH (1:1, 10 mL) was added K_2CO_3 (1.38 g, 10 mmol). The reaction mixture was stirred at rt for 2 h

and filtered at reduced pressure. Then H_2O was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over Na_2SO_4 and the solution was used directly for next step.

A solution of CuCl (49 mg, 0.50 mmol) and TMEDA (150 μL, 1.0 mmol) in acetone (3 mL) was bubbled with air for 10 min at rt, then the above solution was added and continued to stirred with air at rt for 4 h, aqueous NH_4Cl was added to quench the reaction. The mixture was extracted with CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and then concentrated at reduced pressure. The residue was subjected to chromatography to give compound **Ⅱ-2134** (100 mg, 55%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 7.70 (d, 2H), 7.65 – 7.53 (m, 5H), 7.49 (t, *J* = 7.5 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 152.84, 142.68, 134.93, 134.93, 133.78, 133.78, 131.46, 129.52, 129.52, 119.38, 118.37, 118.37, 111.68, 80.32, 80.06, 79.13, 73.99, 73.24, 67.26, 66.90, 64.67, 64.13, 28.46, 28.46, 28.46. ESI-MS: calcd for $C_{25}H_{19}NO_2$ $[M]^+$ 365.14103, found 365.15813.

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